

# The combined use of steam-treated bentonites and natural zeolites in the oenological refining process

M. MERCURIO<sup>1,\*</sup>, D. L. BISH<sup>2</sup>, P. CAPPELLETTI<sup>3</sup>, B. DE GENNARO<sup>4</sup>, M. DE GENNARO<sup>5</sup>, C. GRIFA<sup>1</sup>, F. IZZO<sup>1</sup>, V. MERCURIO<sup>6</sup>, V. MORRA<sup>3</sup> AND A. LANGELLA<sup>1</sup>

<sup>1</sup> Dipartimento di Scienze e Tecnologie, Università degli Studi del Sannio, Via dei Mulini 59/A, 82100 Benevento, Italy

<sup>2</sup> Department of Geological Sciences, Indiana University, Bloomington, IN 47405, USA

<sup>3</sup> Dipartimento di Scienze della Terra, dell'Ambiente e delle Risorse (DiSTAR), Università di Napoli Federico II, Via Mezzocannone 8, 80134 Naples, Italy

<sup>4</sup> Dipartimento di Ingegneria Chimica, dei Materiali e della Produzione Industriale (DICMAPI), Università di Napoli, Federico II, P.le Tecchio 80, 80125 Naples, Italy

<sup>5</sup> INNOVA SCaRL, Via Campi Flegrei 34, 80078, Pozzuoli (Naples), Italy

<sup>6</sup> Winemaker, Salita Santa Croce 27, 80053 Castellammare di Stabia (Naples), Italy

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## ABSTRACT

Industrial minerals, particularly bentonites, have long been used in treatments to improve the stability and shelf life of white wines. We evaluated a new combination of rocks and minerals, including steam-treated bentonites and natural zeolites (chabazite and phillipsite), to greatly reduce the risk of protein and tartaric instability of wines. Detailed mineralogical, chemical and electrokinetic studies of these materials were conducted using powder X-ray diffraction (PXRD), X-ray fluorescence (XRF), microporosimetry, BET surface-area analysis and zeta-potential measurements. Several model wine solutions containing Bovine Serum Albumin (BSA) were prepared to evaluate the oenological performance of the rock/mineral combinations. UV-VIS spectrophotometry and ion chromatography were used to evaluate the degree of wine stabilization from the protein and tartaric point of view.

The experimental results showed that steam treatment modifies both the microporosity and external surface area of the bentonite. These changes in surface area, along with creation of hydrophobic surfaces, significantly modified the behaviour of the steam-treated bentonites, requiring an increase in the amount of material necessary to bring the protein content to required levels. An important benefit derived from the use of steam-treated bentonites is that the pre-mixing with water before addition to wine is not necessary, as the material is readily dispersed. Finally, the addition of natural zeolites effectively decreased the potassium content, thereby improving the tartaric stability of white wines. In addition, this procedure results in minimal waste, as the bentonite-zeolite mixture can be reused as soil amendments in agriculture.

**KEYWORDS:** natural zeolite, protein stability, steam-treated bentonite, tartaric stability, white wine.

## Introduction

STORAGE and transportation of white wines have always represented a significant problem in the oenological industry, as these aspects of wine

production require a suitable wine colloidal stability (Ribéreau-Gayon *et al.*, 2006). Turbidity and deposit formation for these wines are due to the spontaneous denaturation and flocculation of thermosensitive proteins occurring as colloidal suspensions (Mercurio *et al.*, 2010). Their precipitate is known as protein *Casse*, which is linked primarily to inappropriate storage temperature and pH changes (Mesquita *et al.*, 2001). Proteins bound

\*E-mail: mariano.mercurio@unisannio.it

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to the *Casse* derive essentially from the grapes and are produced by the plant to give protection from pathogens (Waters *et al.*, 1996; Hayasaka *et al.*, 2001). Haze-responsible proteins (Pathogenesis-related (PR), proteins) are thaumatin-like (TL) protein and chitinases, both deriving from grape berries (Waters *et al.*, 1996, 1998). The maximum acceptable total protein content of a wine is generally fixed at ~275 mg/l (Bayly and Berg, 1967; Moretti and Bert, 1965; Moio and Addeo, 1989). Some proteins may be more stable in a particular wine than in others, depending on factors such as pH, ionic strength, ethanol concentration, temperature, as well as the content of phenolics, tannins and cations (typically copper). The purpose of stabilizing agents (fining agents), regardless of the type of protein, is to remove excess proteins from the wine without compromising the quality of the final product. Protein dimensions and their high solubilities make it difficult to remove these components by simple filtration (Eisenhour and Brown, 2009). This problem has been tackled over the past century using natural rocks, namely bentonites characterized by a high content (~80 wt.%) of dioctahedral smectite. The use of bentonite currently represents the most efficient procedure for processing white wines, only slightly affecting the quality of the product (Lambri *et al.*, 2010). The application of bentonite (Saywell, 1934; Blade and Boulton, 1988; Achaerandio *et al.*, 2001; Ribéreau-Gayon *et al.*, 2006) ultimately resulted in resolution OENO 11/2003 of the 'Organization Internationale de la Vigne et du Vin' (OIV, 2003), which defines the chemical, mineralogical and technological requirements of a bentonite if it is to be suitable for application in the wine industry.

Applications of natural materials of potential industrial interest generally focus on establishing the primary mineralogical and technological features of the raw materials using a range of analytical methods common in mineralogy and material science. Following these methods, Mercurio *et al.* (2010) evaluated the use of natural zeolites (chabazite and phillipsite) as protein stabilizers for some white wines (*Fiano di Avellino* and *Greco di Tufo* as *DOCG* – 'Controlled designation of origin guaranteed'; *Falanghina del Sannio* as *DOC* – 'Controlled designation of origin') from the Campanian Region (Southern Italy). Their studies demonstrated that in some instances, the use of natural zeolites improved protein stabilization and facilitated a decrease in potassium, thereby improving tartaric stability. The results also suggested the possibility of using other geomaterials.

It is also known that such applications of minerals have the potential to cause undesirable side effects (Catarino *et al.*, 2008; Lambri *et al.*, 2010). In fact, the organoleptic quality of the final product can be influenced greatly by the particular stabilizing treatment. As in the protein adsorption process, important interactions may occur with other components linked to the wine matrix, which may thereby enhance or degrade the development of excellent aromatic qualities. For these reasons, and given the importance of these longstanding problems, there has been continued screening of potential components and methodologies that can replace or at least augment the conventional processes (i.e. Sarmento *et al.*, 2000; Marchal, 2010).

It is well known that natural systems such as those in which bentonite deposits occur are commonly chemically, physically and mineralogically heterogeneous. For these reasons, careful selection of appropriate homogeneous geomaterials, from suppliers who monitor the required properties is crucial in order to avoid the use of batches with features different from those required. Indeed, the literature refers to cryptic variations in bentonite deposits, namely structural variations linked to composition or layer charge (e.g. Meunier *et al.*, 2004; Christidis and Makri, 2007). These authors demonstrated that in many important bentonite deposits, such as those from Charente (France) and Milos (Aegean, Greece), the  $(Al_2O_3 + Fe_2O_3)/MgO$  ratio in smectites decreases from the core to the boundaries of the deposits, with a concomitant increase in cation-exchange capacity (CEC). Both of these variations may have significant effects on the performance of bentonite in wine production.

The present study focused on these aspects and, in particular, it includes full characterization of the minerals (including partial or unknown crystal structure mineral content) occurring in the bentonite samples used in oenological treatments. In addition, the research evaluated possible variations in the surface properties of bentonite-forming minerals, such as smectites after steam treatment, which has been shown to greatly reduce the osmotic swelling property of smectite and to modify the surface and electrokinetic properties (Couture, 1985; Oscarson and Dixon, 1989; Bish *et al.*, 1999; Heuser *et al.*, 2014). To date, the only study of the application of steam-treated bentonite in the oenological sector was carried out by Sarmento *et al.* (2000). However, their material was not characterized and the only information available on the bentonite was that provided by the supplier. These authors concluded that treated bentonite was

not particularly effective in the protein adsorption process.

Tartaric instability, mainly due to the crystallization of potassium tartrate salts ( $C_4H_5KO_6$ ), is an additional important phenomenon impacting on the wine production process, and several methods have been proposed for improving the tartrate stabilization of wine (Ribéreau-Gayon *et al.*, 2006). The methods most commonly applied in the wine industry are (1) a chemical-physical method requiring addition of a metatartaric acid; (2) a physical method involving prolonged cold stabilization; and (3) passage of wine through a column containing a cationic or anionic resin. However, all three of these methods have important deleterious side effects (e.g. colder temperatures increase the wine's ability to absorb oxygen which leads to premature aging). More details can be found in Ribéreau-Gayon *et al.* (2006).

Thus, this present study was extended to evaluate the feasibility of coupling steam-treated bentonites and natural zeolites, known as excellent ion exchangers for  $K^+$ , in a single treatment to obtain improvements in both protein and tartaric stabilization, respectively.

## Experimental

### Raw materials

#### *Commercial bentonites in oenological processes*

The distinction between smectite and bentonite is commonly disregarded, particularly in the commercial and industrial literature. The term bentonite was proposed originally by Knight (1898) for a Cretaceous clay deposit in Fort Benton (Wyoming, USA), and it is currently used as a rock term to describe "...*natural clay materials that are composed primarily of the clay mineral smectite...*" (Eisenhour and Brown, 2009; Güven, 2009). Smectite-group minerals are hydrous and expandable 2:1-type layer silicates and their classification (natural and synthetic) is reported by Güven (2009) and references therein. Smectites are generally classified as dioctahedral smectites, containing mainly trivalent cations (Al, Fe) in two out of three octahedral sites, and trioctahedral smectites, where most or all three octahedral sites are occupied by divalent cations (namely Fe, Mg). Whenever the primary octahedral cation is  $Al^{3+}$ , with the majority of the layer charge arising from tetrahedral Al-for-Si substitution, the mineral is known as montmorillonite, the most common mineral in bentonite deposits (the term montmorillonite was introduced by Cronstedt (1758) and was

later used by Damour and Salvétat (1847) for a deposit close to Montmorillon, France).

From a commercial perspective, two main types of bentonites exist, namely Na- and Ca-bentonites. The first type displays an ability to adsorb and retain large quantities of water and to swell osmotically. In contrast, the second type displays lower water adsorption and swelling capacity and has limited osmotic swelling ability. For these reasons, different types of bentonite are suitable for different specific applications, including metal casting, pet-waste adsorbents, drilling fluids, iron-ore pelletizing, bleaching and clarifying, desiccants, papermaking, environmental sealants and civil engineering. Sodium-rich bentonites also play a major role in the protein stabilization of white wines (OIV, 2003).

Montmorillonites have numerous technologically important properties. In particular, they can completely delaminate in some aqueous solutions (exhibiting hydrophilic behaviour), thereby offering a very large surface area for interaction with other molecules and dissolved ions. This property, and the tendency of some montmorillonites to swell osmotically and form gels, constitute the primary reasons bentonites must be dispersed in an aqueous colloidal suspension before being contacted with wine.

The present research reports on the characterization and evaluation of ten commercial bentonite mixtures currently used in oenological processes and distributed in Italy. For the sake of confidentiality, different abbreviations for these commercial samples have been used. According to technical data (provided by three suppliers), all samples satisfy the OENO 11/2003 resolution ("excellent ability to swell in water"). Thus, no treatments or purification procedures were carried out on the samples that were characterized for chemical, physical and mineralogical parameters.

#### *Natural zeolites*

A sample of the yellow facies of zeolitized Campanian Ignimbrite (Langella *et al.*, 2013; Mercurio *et al.* 2014) was also tested in combination with steam-treated bentonite, as it has been demonstrated previously that natural zeolites (chabazite and phillipsite) can be used advantageously to solve oenological problems. In particular, Mercurio *et al.* (2010) performed a detailed analysis of the performance of this material, demonstrating its ability to remove  $K^+$  ions from wines, thereby increasing their tartaric stability. The sample investigated had a <2 mm grain size, a total zeolite content of ~56 wt.%

(26 wt.% chabazite, 30 wt.% phillipsite), 5 wt.% smectite, 16 wt.% feldspar and traces of biotite. The cation-exchange capacity (CEC) was 2.05 mEq/g, and the external cation-exchange capacity (ECEC) was 0.157 mEq/g.

### Analytical procedures adopted for bentonites

#### Steam treatments

Steam treatments (Bish *et al.*, 1999) were carried out at Indiana University (Dept. of Geological Sciences) in 23 ml Teflon-lined Parr acid digestion bombs (Parr Instrument Company, Part # 4749). For all samples, 0.34 g of Milli-Q water (Millipore Corporation®) and 4.84 g of bentonite were used, and the sealed vessels were heated at 250°C for seven days. After cooling (24 h), treated powders were stored in screw-cap plastic bottles at room temperature until use.

#### Quantitative mineralogical PXRD analysis and XRF whole-rock chemical analysis

Approximately 2 g of each sample were used for powder X-ray diffraction (PXRD) analysis. The material was crushed by hand in an agate mortar, and an  $\alpha$ -Al<sub>2</sub>O<sub>3</sub> internal standard (1  $\mu$ m, Buehler Micropolish, MSI 300 C) was added to each sample in an amount of 20 wt.%. The PXRD patterns were measured at Indiana University (Dept. of Geological Sciences) using a Bruker D8 Advance X-ray diffractometer equipped with incident- and diffracted-beam Soller slits, a SolX solid-state Si(Li) energy-dispersive detector and CuK $\alpha$  radiation (45 kV, 35 mA). Data were measured from 20–70°2 $\theta$  using 0.02° steps and 2 s/step count time. Relative humidity was measured inside the diffractometer enclosure using a Control Company Traceable Hygrometer (model 4185). Quantitative mineralogical analyses were performed using combined Rietveld (Bish and Post, 1993) and reference intensity ratio (RIR) methods by means of *TOPAS 4.2* software (BRUKER AXS GmbH). Atomic starting coordinates for identified crystalline phases were taken from the literature (Inorganic Crystal Structure Database, ICSD, 2014) and included the following: quartz (Le Page and Donnay, 1976), biotite (Takeda and Ross, 1975), gypsum (Knight *et al.*, 1999), clinoptilolite (Cappelletti *et al.*, 1999), kaolinite (Bish and Von Dreele, 1989), albite (Prewitt *et al.*, 1976), sanidine (Phillips and Ribbe, 1973) and cristobalite (Downs and Palmer, 1994). Preferred orientation was treated, whenever needed, for each phase with the March-Dollase approach (Dollase,

1986). Phases with partial or unknown crystal structures were quantified by adding a ‘peaks phase’ with the *TOPAS* software.

Chemical analyses were obtained by X-ray fluorescence (XRF) spectrometry using a Panalytical AXIOS-Advanced spectrometer (Rh tube) at Bari University following the methods described by Franzini *et al.* (1972) and Acquafredda *et al.* (1999). Relative uncertainty was within  $\pm 1\%$  relative for SiO<sub>2</sub>, TiO<sub>2</sub>, Al<sub>2</sub>O<sub>3</sub>, Fe<sub>2</sub>O<sub>3</sub> (total), CaO, K<sub>2</sub>O and MnO;  $\pm 4\%$  relative for MgO, Na<sub>2</sub>O and P<sub>2</sub>O<sub>5</sub>. Loss on ignition was measured as weight loss after firing at 1100°C.

#### Cation-exchange capacity (CEC), anion-exchange capacity (AEC) and specific surface area (SSA) evaluation

The batch exchange method (BEM) (Cerri *et al.*, 2002), carried out at FEDERICO II University (DiSTAR), was used to measure CEC values for all samples by extracting Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup> and Ca<sup>2+</sup> cations with a solution of ammonium chloride (Aldrich, assay 99.5%, CAS [12125-02-9]). The method consisted of placing 1.0 g samples in Nalgene tubes with 35 ml of 1 M NH<sub>4</sub>Cl solution under continuous stirring at a constant temperature of  $\sim 60^\circ\text{C}$ . The exhausted solution was separated from the solids every two hours by centrifugation and was replaced with the same volume of a fresh solution. At the end of the exchange, the concentrations of cations released were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES), yielding the CEC, in mEq/g. Ten repetitions of the exchange treatment were usually required to attain  $< 0.1$  mg/l cation concentration in the exchange solution.

The AEC of bentonites (FEDERICO II University, DICMAPI) was estimated by contacting 1.0 g of these samples for three days at 25°C under continuous stirring with 30 ml of 100-mM KCl and/or KNO<sub>3</sub> solutions. Three different replicates were prepared for each exchange, and the solutions were changed and recovered every 24 h. Anion concentrations were analysed via high-pressure liquid chromatography (HPLC).

Microporosimetric and SSA measurements (FEDERICO II University, DICMAPI) were carried out using N<sub>2</sub> adsorption at 77 K using a Micromeritics ASAP 2020 volumetric instrument. Samples were degassed at 473 K for four hours prior to analysis. Data analysis used *Physi ViewCalc 1.0* (Micromeritics, freeware), which facilitated determination of the SSA by means of the Langmuir method, Brunauer-Emmett-Teller

(BET) method and t-plot model (Gregg and Sing, 1982). However, the most reliable surface area values were calculated using the BET equation along with the  $C$  and the  $Q_m$  values. Here  $C$  is a constant and, according to Dollimore *et al.* (1976), unusually large values are an indication of the presence of micropores. The second parameter ( $Q_m$ ) represents the monolayer capacity, i.e. the amount of gas adsorbed when a uniform single molecular layer on the sample surface is formed. These results were combined with a t-plot model (Gregg and Sing, 1982) to identify the contribution to SSA of the fraction linked to microporosity and to the external porosity.

Due to its pronounced tendency to gel, it was not possible to measure these parameters (CEC, AEC and SSA) on sample BT7.

#### Zeta potential and pH

The zeta ( $\zeta$ ) potential has a significant role in clarifying the adsorption mechanism(s) of inorganic and organic species at the untreated/steam-treated bentonite-solution interface. Electrokinetic properties, in general, determine the flotation, flocculation and dispersion properties in suspension systems and provide information about the clay particles, their interactions with the surrounding medium and the electrical properties of particles. Higher  $\zeta$ -potential values provide more stability of the colloidal systems as they originate from electrostatic repulsive forces that hinder particle aggregation. Low  $\zeta$ -potential values indicate that attractive forces prevail over repulsive forces, thereby facilitating processes such as coagulation and flocculation.

To prepare the samples 0.25 g of both untreated and steam-treated bentonites were gently ground in an agate mortar. Then 50 ml of deionized water was added to this powder and the solution was blended in a Waring laboratory blender for five minutes and then left to settle for one hour. The pH was measured on all the bentonite suspensions. Solutions were then filtered using a 0.45  $\mu\text{m}$  syringe filter and were analysed for  $\zeta$ -potential with a Malvern Zetasizer Nano ZS using an electrophoretic light-scattering technique along with non-invasive backscatter optics and a 633 nm laser (Dept. of Chemistry, Indiana University).

#### Protein and tartaric stability

In order to test the capability of bentonites to adsorb protein, model wine solutions having 12% (v/v) ethanol (Fluka, assay  $\geq 99.8\%$ , CAS [64-17-5])

and 2 g/l of potassium hydrogen tartrate (Aldrich, 99% assay, CAS [868-14-4]) in distilled water were produced (DICMAPI; University of Sannio, Dept. of Science and Technology). The final pH of the solution was 3.7. To ensure complete suspension of the bentonites, the hydrophilic untreated bentonites (solid to liquid ratio: 50:750 mg/l) were mixed with 150 ml of model wine solution by stirring continuously for two hours using magnetic stirrers.

In contrast, hydrophobic steam-treated bentonites suspended immediately (solid to liquid ratio: 50:2000 mg/l) when added directly to model wine solutions without the use of significant mechanical agitation. Subsequently, 150 ml of a solution of 1000 ppm of Bovine Serum Albumin (BSA) (Aldrich fraction V,  $\geq 96\%$  assay, CAS [9048-46-8]) was added to the model wine solution, giving a final concentration of 500 ppm of BSA. The solution was further stirred for 30 min in order to ensure equilibrium, according to Blade and Boulton (1988). Suspended solids were then separated by centrifugation at 5000 rpm for five min, and BSA was analysed using a UV/VIS spectrophotometer (HITACHI U-2000) at 280 nm wavelength. The use of BSA for evaluating the uptake behaviour of the bentonites selected is related to several specific features, particularly its high molecular weight (66 kDa). If the testing material is able to adsorb this molecule, then it will generally adsorb all proteins having lower molecular weight, such as those usually found in wines (Sarmiento *et al.*, 2000 and references therein). Our experimental procedure was developed based on this premise.

To evaluate the ability of this experimental procedure to stabilize a 'real' wine, heat tests were conducted, a common and reliable procedure used in the oenological sector. These experiments consisted of mixing untreated and steam-treated bentonites with 300 ml of unstabilized wine. Solutions were kept for two hours in an oven at 90°C, followed by two additional hours of treatment at 4°C. Samples were then held at ambient temperature ( $\sim 20^\circ\text{C}$ ). Turbidity was measured in nephelometric turbidity units (NTU), before and after the oven treatment, using a Hanna Instruments HI83749 turbidimeter. The test is based on the fact that proteins denature during the heat treatment, thereby creating turbidity. A result was judged to be positive (wine stabilized) whenever the difference in terms of NTU, before and after heat treatments, was  $< 2$  (Sarmiento *et al.*, 2000).

After protein stabilization, the model wine solution underwent a further step, aimed at mitigating the precipitation of potassium tartrate, by means of a

TABLE 1. Quantitative PXRD analysis of selected samples of bentonite used in the oenological sector. Sme = dioctahedral smectite, Crs = Cristobalite, Gp = Gypsum, Qtz = Quartz, Cal = Calcite, Ab = Albite, Kfs = K-feldspar, Mca = Mica, Kln = Kaolinite, Cpt = Clinoptilolite, tr.<1%.

Untreated bentonite samples	Mineral phases (wt.%)											Total
	Sme*	Opal CT	Crs	Gp	Qtz	Cal	Ab	Kfs	Mca**	Kln	Cpt	
BT1	89 (±4)			tr.	5 (±1)	tr.	2 (±1)	2 (±2)	1 (±1)			99
BT2	98 (±4)		tr.		tr.		1 (±1)		tr.			99
BT3	90 (±4)		1 (±1)				3 (±1)	5 (±2)	tr.			99
BT4	81 (±4)	9 (±2)	3 (±1)	tr.	1 (±1)		2 (±1)	1 (±1)	tr.	tr.	1 (±1)	98
BT5	86 (±4)		4 (±1)				5 (±1)	4 (±2)	1 (±1)			100
BT6	78 (±4)		3 (±1)		4 (±1)		3 (±1)	7 (±2)	3 (±1)	2 (±1)		100
BT7	85 (±4)				1 (±1)		8 (±1)	2 (±2)			4 (±1)	100
BT8	67 (±4)	8 (±2)	9 (±1)	1 (±1)	8 (±1)		1 (±1)	2 (±2)	1 (±1)		3 (±1)	100
BT9	89 (±4)		3 (±1)		1 (±1)	tr.	2 (±1)	2 (±2)	tr.		1 (±1)	98
BT10	84 (±4)		9 (±1)	tr.	1 (±1)		2 (±1)	2 (±2)	tr.	1 (±1)	tr.	99

\*Peaks phase method of *TOPAS* (see reference therein), representing partial or unknown crystal structure material content.

\*\*Phases with 10 Å basal repeat distance (illite/muscovite/biotite).

Errors in parentheses. tr. = trace

natural zeolite-rich rock, the yellow facies of Campanian Ignimbrite described above. Two g/l of this zeolite-rich tuff were added to the protein-stabilized model wine solution. The potassium content in solution was evaluated by ion chromatography (DIONEX DX 120 equipped with a CS12A cationic column and an isocratic elution system).

## Results and discussion

To assess and predict the performance of a rock/mineral mixture for industrial applications, it is mandatory to understand the sample mineralogy. It is also important to evaluate potential mineralogical changes induced by mineral processing (e.g. steam treatment) or by the industrial application itself.

### Mineralogical analysis of bentonites

Results of the mineralogical analyses of the selected bentonites are reported in Table 1. All bentonite samples contained >80 wt.% dioctahedral smectite, with the exception of samples BT6 and BT8 (78 and 67 wt.%, respectively); other ubiquitous phases were feldspars and mica (few wt.%). Quartz was detected in almost all samples, except for BT3 and BT5, and its content ranged

between 1 (samples BT4 and BT9) and 7 wt.% (BT8). Cristobalite occurred in almost all samples, up to ~9 wt.% in samples BT8 and BT10. Clinoptilolite-group zeolites were detected in five samples, but their content never exceeded 4 wt.%. Kaolinite (BT4, BT6 and BT10), gypsum (BT1, BT4 and BT8) and calcite (BT1) were also found in trace amounts. The presence of opal-CT in sample BT4 and BT8 was detected easily by the occurrence of a characteristic broad hump in the 21.4–21.9°2θ region of the PXRD pattern, and its content (8–9 wt.%) was modelled using a Lorentzian strain-broadened cristobalite pattern (Peacor, 1973). Most of these phase associations are typical of the minerogenetic evolution of an acid glass (rhyolite-rhyodacite) precursor of volcanic origin (Cerri *et al.*, 2001).

In order to assess the effects of steam treatments, PXRD data were also measured for steam-treated samples using identical measurement conditions. As reported by Bish *et al.* (1999) and Zhu (2009), the 001 reflections of smectites were broader for steamed samples (at a given relative humidity), but other than the removal of gypsum in steam-treated samples BT1, BT4, BT8 and BT10, no other changes were detected by PXRD (Fig. 1). Gypsum and calcite may suggest diagenetic processes involving additional fluids.

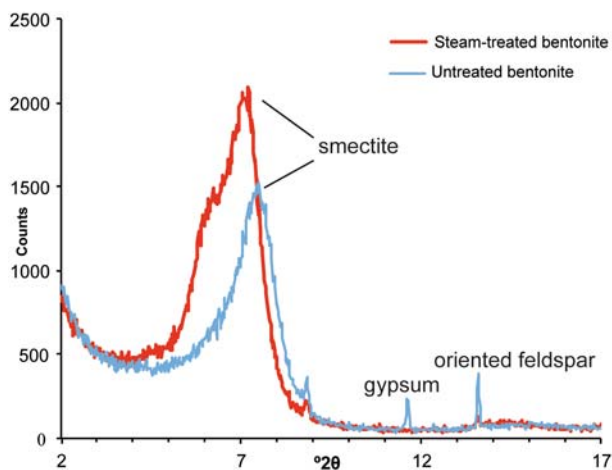


FIG. 1. Representative PXRD patterns illustrating differences between untreated and steam-treated bentonite.

### Chemical analysis of bentonites

Table 2 reports the composition of the untreated bentonites investigated: SiO<sub>2</sub> ranged from 49.2 to 67.5 wt.% (samples BT2 and BT7, respectively), Al<sub>2</sub>O<sub>3</sub> had its lowest value in sample BT4 (12.6 wt.%) and reached 21.66 wt.% in sample BT5. Sample BT2 displayed the highest content of divalent cations (CaO + MgO = 5.62 wt.%) and the lowest concentration of Na<sub>2</sub>O + K<sub>2</sub>O (1.25 wt.%), along with the highest LOI value (22.7 wt.%). With the exception of BT6, all samples showed Na<sub>2</sub>O contents >1 wt.%, the greatest being in sample BT3 (3.64 wt.%). Comparison between these chemical data and the literature values for typical bentonites is fruitless, due to the considerable

mineralogical variability exhibited by these rocks (see Ahonen *et al.*, 2008; Karnland *et al.*, 2006; Christidis, 2006 and references therein). Marked chemical inconsistencies were apparent, even among the samples investigated, which were expected based on the significant mineralogical variability exhibited by these samples. These data show that none of the analysed bentonites were mainly in the sodium or calcium form.

### CEC and AEC evaluation

Table 3 lists the CEC values measured on the selected bentonites. No substantial differences were observed between the steam-treated and untreated

TABLE 2. Compositions of the bentonites investigated from XRF data (wt.%).

	BT1	BT2	BT3	BT4	BT5	BT6	BT7	BT8	BT9	BT10
SiO <sub>2</sub>	55.43	49.21	52.18	65.11	53.03	52.20	67.50	65.34	65.33	58.85
Al <sub>2</sub> O <sub>3</sub>	18.65	18.23	21.18	12.60	21.66	20.77	13.45	12.81	13.35	16.46
TiO <sub>2</sub>	0.14	0.21	0.18	0.21	0.20	0.29	0.16	0.19	0.16	0.20
Fe <sub>2</sub> O <sub>3</sub>	3.74	2.70	2.19	1.79	2.46	3.56	1.27	1.73	1.25	2.19
MnO	0.02	0.06	0.01	0.01	0.01	0.03	0.02	0.01	0.03	0.01
MgO	2.64	4.23	2.70	2.33	2.64	3.16	2.41	2.71	2.38	2.74
CaO	1.11	1.39	1.22	0.73	1.19	1.37	1.02	0.86	1.07	0.95
Na <sub>2</sub> O	2.06	1.06	3.64	2.08	2.43	0.99	2.62	1.33	1.07	3.44
K <sub>2</sub> O	0.38	0.19	0.77	0.52	0.85	1.11	0.61	0.60	0.55	0.64
P <sub>2</sub> O <sub>5</sub>	0.03	0.02	0.02	0.01	0.02	0.03	0.43	0.22	0.01	0.02
LOI*	15.80	22.70	15.90	14.60	15.50	16.50	10.50	14.20	14.80	14.50

LOI\*: loss on ignition.

TABLE 3. Cation-exchange capacity of the untreated and steam-treated bentonite samples.

Bentonite samples	Untreated CEC (mEq/g)	Steam-treated CEC (mEq/g)
BT1	1.19	1.03
BT2	1.11	1.27
BT3	1.57	1.37
BT4	1.15	0.95
BT5	1.41	1.19
BT6	0.94	1.12
BT8	0.94	1.08
BT9	0.96	1.00
BT10	1.56	1.45

samples (CEC range for untreated samples: 0.94–1.57 mEq/g; CEC range for steam-treated samples: 0.95–1.45 mEq/g), consistent with previous results by Couture (1985) and Oscarson *et al.* (1994). AEC values (not reported) were all below the detection limit, demonstrating that both untreated and steam-treated bentonite samples are good cation exchangers but are not useful as anion exchangers. These results confirm that the changes induced by steam treatment did not significantly influence the exchange properties of the contained smectites.

### Specific surface area evaluation

The surface area values from both the Langmuir and BET methods are reported in Table 4, together with the surface fraction contributions from external and microporous surfaces. All bentonite samples showed a remarkable decrease in surface area after steam treatment. This result was expected, as steam treatment significantly reduced the tendency for the bentonites to interact with water and largely affects the micro- and mesoporosity measured by an N<sub>2</sub>-based porosimetric analysis (Zhu, 2009).

Adsorption isotherms for the entire set of samples exhibited pseudo-type II behaviour, in agreement with Sing and Williams (2005), probably due to the metastability of the adsorbed multilayer and to the low degree of pore curvature and non-rigidity of the aggregate structure. Such systems tend to give isotherms exhibiting an adsorption hysteresis (Fig. 2) Moreover, the adsorption stage of the isotherms typically resembles the type-II category typical of nonporous or macroporous structures. The presence of a

hysteresis between the adsorption and desorption branches, however, modifies the specific classification to pseudo-type II.

The surface area values are reported in Table 4 along with C and the Q<sub>m</sub> values calculated using the BET equation. The value of Q<sub>m</sub>, representing the monolayer capacity, was reduced for almost all steam-treated samples to ~50% of the untreated values, confirming that modification of the bentonite external surface took place. Moreover, although the Langmuir calculations of external surface area have been criticized as being based on an inadequate model (Gregg and Sing, 1982), similar trends persisted when comparing the untreated and steam-treated samples, once again underlining the surface area reduction between untreated and steam-treated samples.

Although the bentonite BET surface areas extracted from composite N<sub>2</sub> isotherms could be skewed by the presence of micropores, the t-plot method (applied in our case with a universal thickness equation) provided an empirical means of extracting the contributions of micropores and external surfaces (last three columns of Table 4). Indeed, the significant contribution of microporosity to the surface area for many untreated (BT1, BT2, BT3, BT5, BT6) and steam-treated (BT2, BT3, BT5, BT6) samples (ranging between 42 (BT5) and 81% (BT3)) was evident; minor contributions were recorded for the other samples (from 8% of untreated BT9 to 34% of BT4).

A possible interpretation of such a marked variation in surface area after steam treatment is related to the three-dimensional structure of these materials. In fact, according to Touret *et al.* (1990), a clay system in the dry state can be described by three structural units and by their arrangement in space, with a specific type of porosity corresponding to each structural unit. The first unit is a 'layer', of which the thickness is ~10 Å with a lateral dimension of up to ~1–2 µm (Touret *et al.*, 1990) in which N<sub>2</sub> penetration is impossible due to the large amount of work needed to expand the layers (Aylmore *et al.*, 1970). The second unit has been called a 'tactoid' and is equivalent to a quasi-crystal system (Quirk and Aylmore, 1971), made up of stacks of elementary layers. The stacking of layers in the smectite quasi-crystals has been found to be turbostratic (Tessier, 1984) and in some cases may lead to the occurrence of slit-shaped micropores on broken edges of the layers (Aylmore *et al.*, 1970; Cases *et al.*, 1992; Neaman *et al.*, 2003). The third unit is an 'aggregate', which consists of tactoids/quasi-crystals. The specific arrangement of



TABLE 4. Major results based on N<sub>2</sub> isotherm analyses according to *Physi ViewCalc 1.0* (Micromeritics, freeware).

Sample	Langmuir				BET			Universal t-plot		
	SSA (m <sup>2</sup> /g)	SSA (m <sup>2</sup> /g)	C	Qm (cm <sup>3</sup> /g STP)	Correlation Coefficient	Micropore SA (m <sup>2</sup> /g)	External SA (m <sup>2</sup> /g)	%SSA*	Micropore V (cm <sup>3</sup> /g)	
BT1	38.55	21.72	431.37	4.989	0.9999	7.296	14.423	50.586	0.0031	
BT1 St	19.19	9.4	264.65	2.159	0.9999	1.525	7.874	19.368	0.00074	
BT2	111.34	65.71	291.99	15.093	0.9999	25.333	40.373	62.747	0.0104	
BT2 St	70.61	41.57	388.62	9.551	0.9999	17.248	24.331	70.889	0.00711	
BT3	78.82	48.01	424.35	11.029	0.9999	21.538	26.473	81.358	0.0089	
BT3 St	28.84	14.83	511.33	3.406	0.9999	4.581	10.245	44.714	0.0018	
BT4	103.44	54.41	266.59	12.499	0.9999	13.82	40.59	34.048	0.0058	
BT4 St	30.8	15.46	253.36	3.551	0.9999	2.395	13.062	18.336	0.0011	
BT5	71.17	41.56	310.1	9.547	0.9999	16.343	25.219	64.804	0.0067	
BT5 St	37.21	20.17	334.13	4.633	0.9999	5.991	14.179	42.253	0.0026	
BT6	67.85	39.85	301.73	9.154	0.9999	14.711	25.137	58.523	0.0061	
BT6 St	36.82	20.21	231.52	4.644	0.9999	6.176	14.039	43.992	0.0026	
BT8	60.72	32.04	221.21	7.36	0.9999	7.698	24.343	31.623	0.0032	
BT8 St	41.52	21.06	208.64	4.84	0.9999	3.955	17.105	23.122	0.0017	
BT9	75.69	39.95	170.42	9.176	0.9999	3.029	36.917	8.205	0.0014	
BT9 St	44.09	21.95	219.42	5.043	0.9999	3.203	18.752	17.081	0.0014	
BT10	50.55	25.82	284.17	5.931	0.9999	5.524	20.293	27.221	0.0024	
BT10 St	25.39	13.39	455.08	3.077	0.9999	3.267	10.128	32.257	0.0014	

St = Steam treated; %SSA\* = Micropore/External.

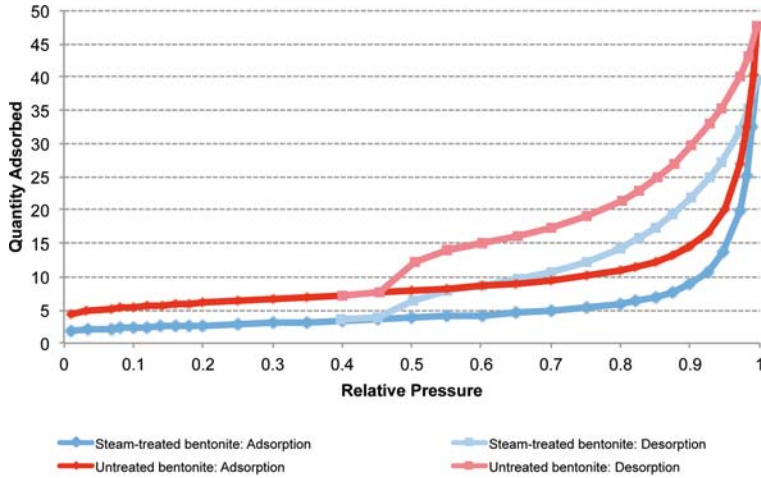


FIG. 2. Representative adsorption isotherms carried out using *ASAP 2020* Micromeritics for untreated and steam-treated bentonite samples.

tactoids/quasi-crystals inside the aggregates results in the presence of small and medium mesopores (i.e. 2–40  $\mu\text{m}$ ) in the inter-tactoid/inter-quasi-crystalline regions (Touret *et al.*, 1990).

Bentonite experienced a significant decrease in surface area during steam treatment; our hypothesis accounts for a variation of surface area not ascribable to the interlayer, which, as stated before, is not affected by  $\text{N}_2$  adsorption but is probably due to a closer proximity between the turbostratic structure and aggregates.

Moreover, although providing additional information that helps interpret the contribution of micropores vs. external surface areas, the t-plot is essentially an empirical method that provides information in terms of micropore volumes by graphical extrapolation. Therefore, results from the t-plot analysis should be considered as only relative

measures of the clay mineral properties (Rutherford *et al.*, 1997). More detailed characterization of the micropore structures could be achieved by exploring the use of different adsorbents (He,  $\text{CO}_2$ , Kr, etc.), which may enhance the evaluation of the surface area.

### Electrokinetic properties

The  $\zeta$ -potential analysis gave very different results for untreated and steam-treated samples, evident in Fig. 3, which reports a single curve displaying a common behaviour for all the materials investigated. In fact, the typical bell-shaped curve (representative for all samples) was narrow before treatment (ranging from  $\sim -50$  to  $\sim 30$  mV) and became broader and less pronounced after steaming

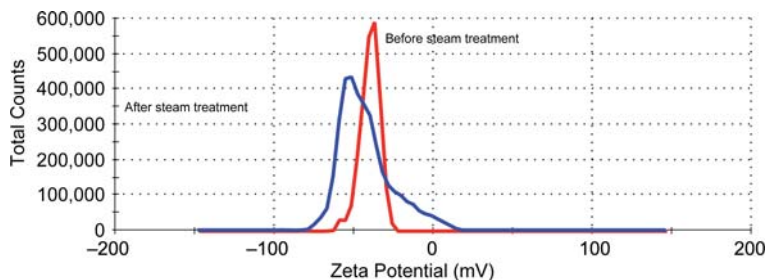


FIG. 3. Representative  $\zeta$ -potential differences between untreated and steam-treated bentonites.

(ranging from  $\sim -78$  to  $\sim 10$  mV). These differences are a consequence of a significant change in the  $\zeta$ -potential that became close to zero or slightly positive in the steam-treated materials.

The pH of the solutions (not reported) always decreased after steam treatment from values above neutral to around 6.5, in agreement with Bish *et al.* (1999). They showed that steam treatment leads to, in the case of polar liquids such as water, an increase in contact angle due to the reduction in the Lewis basicity and therefore to a reduction of the pH.

One of the most important modifications occurring as a result of the steaming process was the conversion of the bentonite behaviour from hydrophilic to hydrophobic. This modification will be explored below as it can be exploited to improve the oenological performance of bentonites.

#### *Oenological performance of untreated and steam-treated bentonites*

Figs 4 and 5 show the results of the interaction between the BSA in the model wine solution (500 ppm) and the selected bentonite adsorbents. The protein adsorption behaviour recorded for untreated and steam-treated samples was quite distinct. The first difference is related to the amount of adsorbent necessary to remove the same amount of BSA (Fig. 4). The solid to liquid ratio required to remove the entire amount of BSA (500 ppm) for untreated samples was  $\sim 750$  mg/l, whereas  $\sim 2000$  mg/l was necessary for the steam-

treated samples. This behaviour is probably due to the previously described decrease in surface area of steam-treated samples (almost halved) after the steam treatment. As the maximum protein amount to be removed from a wine is  $\sim 275$  mg/l, a final amount of 225 mg/l BSA in the model wine solution would require 500 mg/l of hydrophilic (untreated) bentonite and  $\sim 850$  mg/l of hydrophobic (steam-treated) bentonite (Threshold in Fig. 4). However, it is also important to note that the hydrophilic material (untreated bentonite) used in this treatment must be water dispersed before use to achieve a colloidal state. In contrast, the steaming process makes bentonite hydrophobic, and the material can be added to wine with no water pretreatment or significant agitation. This aspect counterbalances the greater solid-to-liquid ratio necessary for steam-treated bentonites to remove the required amount of proteins.

Although their behaviours were different in detail, all steam-treated bentonites removed significant amounts of BSA from the model wine solution (Fig. 5). Samples BT1 and BT8 had the best performances by removing all available BSA (500 ppm), whereas BT6 removed the lowest amount, 275 ppm of BSA, coincidentally the protein content required to be removed for wine stability. Considering all of our data, no relationship was apparent between smectite content and BSA adsorption, suggesting that chemical composition was more important than mineralogy in determining protein uptake.

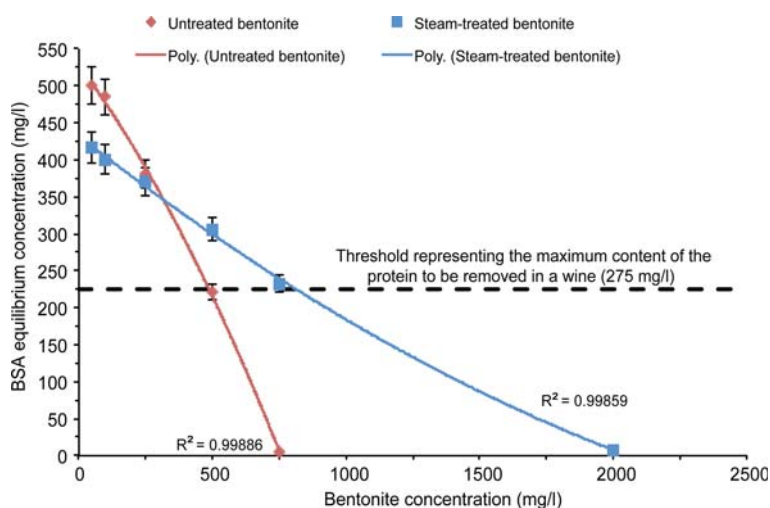


FIG. 4. Representative protein adsorption behaviour for untreated and steam-treated bentonites (error bars  $\pm 5\%$ ); Poly = Polynomial regression trend line (order 2).

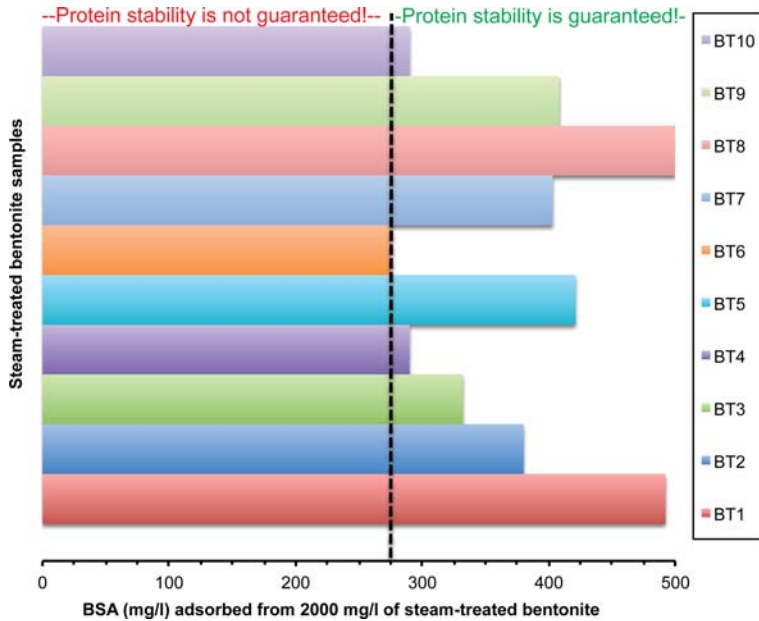


FIG. 5. Protein stability as function of steam-treated bentonite content. The dashed line indicates the maximum protein value recorded in wine (275 mg/l).

Finally, the results of heat tests carried out on samples of white wine, using steam-treated and untreated bentonites, were in full agreement with those obtained on the model wine solutions. The use

of 2 g/l of steam-treated bentonite and 1 g/l of untreated bentonite always gave values of  $\Delta NTU < 2$ , along with a constant clear visual appearance after testing. These results suggest that steam-treated

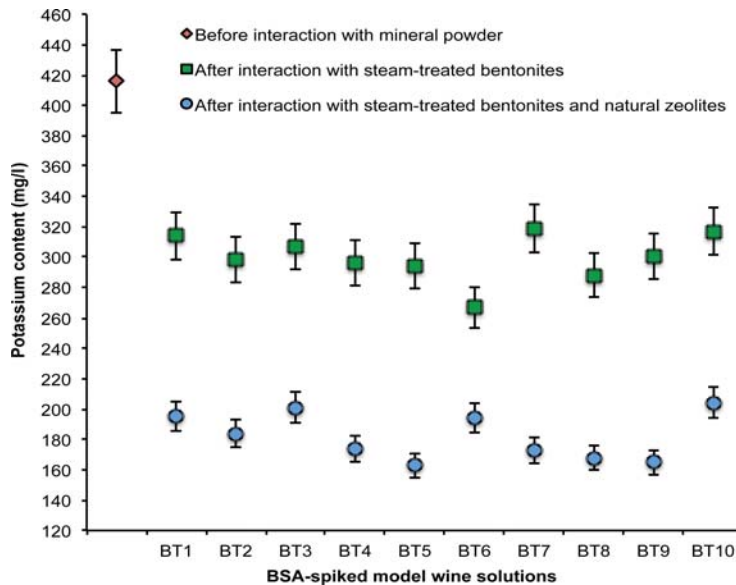


FIG. 6. Potassium contents in BSA-spiked model wine solutions after interaction with steam-treated bentonites and after the combined interaction with steam-treated bentonites and natural zeolite (error bars  $\pm 5\%$ ).

bentonite can be exploited in the adsorption of proteins of white wine.

*Steam-treated bentonites and natural zeolites: towards the definition of a new white wine production step*

Previous studies carried out by Mercurio *et al.* (2010) demonstrated that the use of chabazite- and phillipsite-rich rocks can be useful in the protein and tartaric stabilization of white wines. Although the comparatively large size of natural zeolite crystallites precluded formation of colloidal suspensions, as is common with swelling and hydrophilic clay minerals, we found that an increase in the solid-to-liquid ratio can improve performance. Above all, the high selectivity of zeolites for potassium enables the natural zeolites to contribute to a significant decrease in potassium concentration in wines, thereby improving the

tartaric instability linked to the precipitation of potassium bitartrates. Wyss and Cuénat (2005) demonstrated that a decrease in potassium starting concentration in wines of ~10–30% probably inhibits the precipitation of potassium salts, thereby improving wine stability during transportation and storage. It has been demonstrated already that Campanian zeolitized tuffs (Mercurio *et al.*, 2010) can act as a potassium exchanger, releasing harmless amounts of sodium and/or calcium into solution (wine) during the exchange process. Based on these previous results, samples of model wine solution that had been protein stabilized with steam-treated bentonites were further treated with a zeolitized tuff. Figure 6 shows that this treatment produced significant decreases, up to ~50%, in potassium content. A starting potassium content of 416 mg/l decreased to ~150–200 mg/l after interaction with 2 g/l of natural zeolites. Interaction between natural zeolite and untreated bentonite was

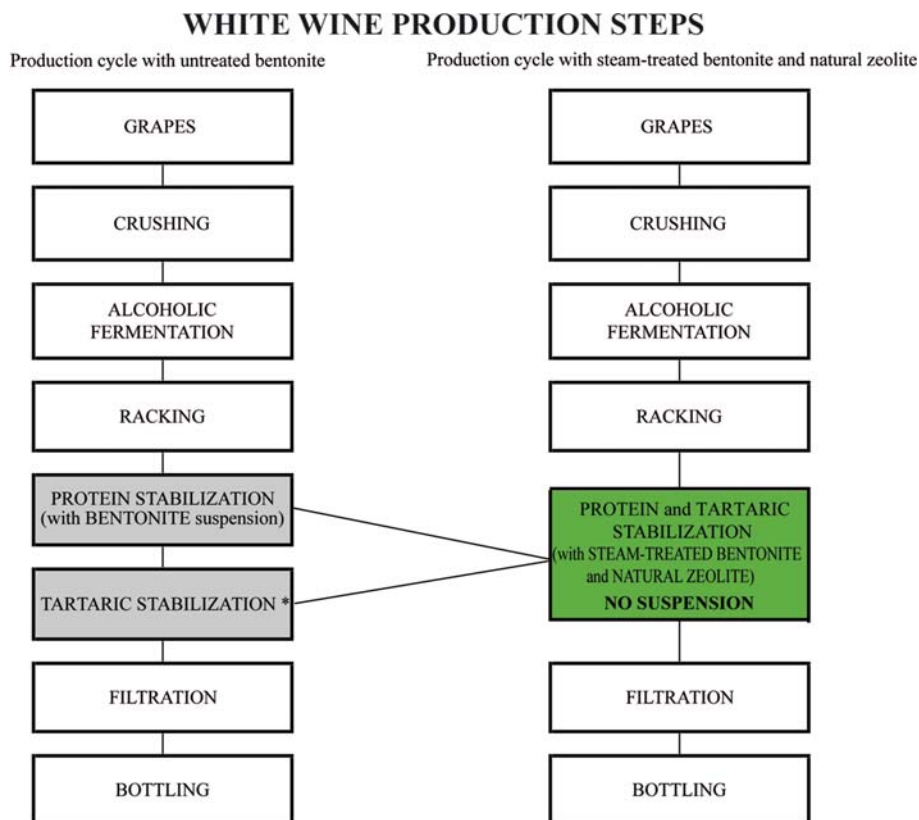


Fig. 7. Comparison between a traditional production cycle using only untreated bentonite (left) and the proposed method using a combination of steam-treated bentonite and natural zeolite (right). \*Addition of a metatartaric acid involving prolonged cold stabilization and passing the wine through a column containing resin in a cationic or anionic form.

not considered due to the hydrophilic behaviour of the latter. This combined approach is expected to be efficient at guaranteeing the tartaric stability of a wine, as the potassium decrease was substantial and consistent for different zeolite samples. These results provide a pathway for a novel step in white wine-making production involving the combined use of steam-treated bentonites and natural zeolitized rocks (zeolite content >50%) prior to bottling, to optimize the protein and tartaric stability of white wines (Fig. 7).

## Conclusions

The present interdisciplinary study evaluated whether surface modifications induced by steam treatments of bentonites can enable these rocks to perform as active agents in the protein stabilization of white wines. Although greater amounts of steam-treated bentonites (~2 g/l) were required for protein stabilization than of untreated bentonite (~0.5–1 g/l), the hydrophobic nature of the former made their application far easier. The protein-stabilized model wine solutions further treated with natural zeolites showed a significant decrease in potassium content, thereby favouring tartaric stability. The results of this study can serve as a potential tool for winemakers, allowing them to optimize the fining agents for specific oenological applications.

The modification of bentonites behaviour from hydrophilic to hydrophobic via steam treatment allows elimination of the preliminary water interaction step that is necessary when using hydrophilic untreated bentonites that gel in water. In contrast, the hydrophobic nature of steam-treated bentonite eliminates the tendency to gel, allowing rapid dispersion of the bentonite in water or wine and favouring the settling of this solid phase.

After interaction with wine, steam-treated bentonite (used to enhance protein stability) and natural zeolites (used to enhance tartaric stability) could be managed directly in the wine cellar, not as a waste to be disposed of but as a raw material to be reused in agriculture. There is an extensive amount of literature on the use of bentonites and natural zeolites in agriculture (Ming and Allen, 2001; Eisenhour and Brown, 2009). Indeed, the mixture of exhausted steam-treated bentonites and exchanged zeolite-rich rocks will be enriched in proteins and potassium, making them valuable as soil amendments, with the additional advantage of virtual elimination of waste-management expenses.

Future research in this area will focus on structural stabilization of the steam-treated bentonites. This

modification may allow bentonites to be used in fixed beds percolated by unstable wines, which could replace the exchange resins currently used to control wine pH and potassium content through hydrogen-potassium exchange. This represents one area for further experiments on a pilot or industrial scale with the active participation of winemakers. To ensure that such an approach could be relevant at the industrial scale, additional organoleptic studies on real wines must be performed to determine the potential effect of these adsorbents on the array of the molecules providing precious flavours to the wine. However, it is well known that zeolitized rocks, as well as groundwater and hydrothermal fluids that may have interacted with them, may contain elevated levels of trace elements, some of which may have deleterious health effects. Thus, further research is required concerning potential releases of such trace elements (e.g. thallium, actinides, etc.) due to interactions between zeolitized rocks and real wine.

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